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An efficient expression vector for stable expression in human liver cells*

(HepG2; human polypeptide chain elongation factor 1α; Geneticin)

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SUMMARY

The expression of the bacterial neomycin resistance (Nm^R)-encoding gene was examined under control of the promoter region of the gene encoding the human polypeptide chain elongation factor 1α (EF-1α). In a human liver cell line, HepG2, the plasmid pEF321-*neo* directed higher expression of the bacterial *neo* gene than the Nm^R gene expression vectors using other promoters, like the SV40 early region or thymidine kinase of Herpes simplex virus and SV40 early region promoter linked to human T-cell leukemia virus-1 enhancer sequences.

It is often difficult to establish human liver cell lines which support the permanent expression of transfected foreign genes. Many mammalian expression vectors which utilize various viral promoters and enhancers have been used in human liver cell lines. In many cases, however, their expression decreases after cell passages even if the high level expression is transiently observed. Especially, the efficiency of colony formation by human liver cells employing drug-resistance genes expressed by various expression vectors is extremely low when compared with other cell types.

Here, we describe a versatile expression vector, pEF321, useful for the establishment of human liver cell clones which permanently express desired genes. The pEF321 vector utilizes the promoter of *EF-1α* gene (Uetsuki et al., 1989). We previously reported that this potent promoter is highly active in almost all kinds of mammalian cells tested. They included not only fibroblasts but also lymphoid or neuronic cells (Kim et al., 1990). A plasmid pEF321-*neo* was constructed by inserting the bacterial Nm^R gene under the EF-α promoter of pEF321 vector (Kim et al., 1990). In this study, we compared the efficiency of its colony formation with those of pSV2-*neo*, pTK-*neo* (or pKan2), pSRα-*neo* plasmids in human hepatoblastoma cell line, HepG2, in the presence of G418. The plasmids pSV2-*neo*, pTK-*neo* (or pKan2), pSRα-*neo* contain the simian virus (SV40) early region promoter, *TK* promoter of herpes simplex virus type 1 (Yates et al., 1984), SV40 early region promoter linked to human T cell leukemia virus-1 enhancer sequences (Takebe et al., 1988), respectively. As shown in Fig. 1 and Table I, the number of colonies formed by pEF321-*neo* was about 100-fold larger than those by pSV2-*neo* or pTK-*neo*, and about tenfold larger than pSRα-*neo*. The average size of colonies formed by pEF321-*neo* plasmid was also severalfold larger than that by other plasmids tested in this study. Our previous Northern blotting data suggested that larger cell colonies reflected higher expression of the transfected genes (data not shown). By the transfection of 1 μg of pEF321-*neo* by Ca·phosphate coprecipitation method in HepG2 cells grown on a 6-cm

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*On request, the authors will supply detailed experimental evidence for conclusions reached in this Brief Note.

Abbreviations: EF-1α, human polypeptide chain elongation factor 1α; *EF-1α*, gene encoding EF-1α; G418, Geneticin; Nm, neomycin; ^{R}, resistance/resistant; SV40, simian virus 40; *TK*, thymidine kinase-encoding gene.

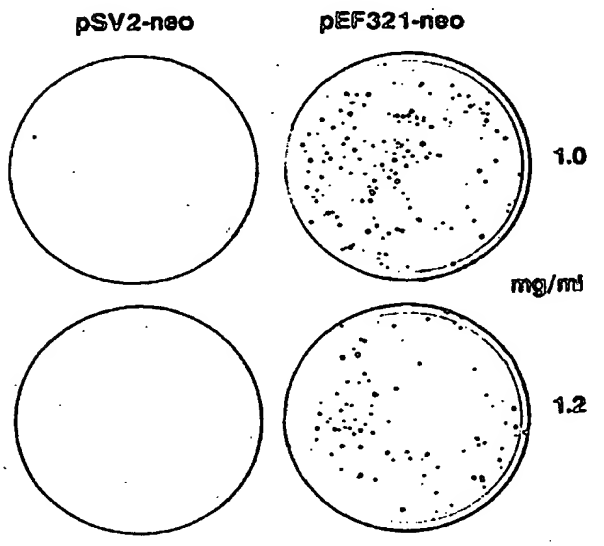

Fig. 1. Colony formation by HepG2 cells (1.6×10^6) transfected with 1 mg of plasmids pSV2-*neo* or pEF321-*neo* on 6 cm-plates. After 24 h, 3×10^5 cells from each culture were transferred to 10 cm plates. After selection by 1.0 or 1.2 mg G418/ml in Dulbecco's modified Eagle's medium with 10% fetal bovine serum for four weeks, colonies were stained with 5% Giemsa solution.

TABLE I

Number of colonies[a] formed in HepG2 cells

Plasmid[b]	G418 (mg/ml)	
	0.8	1.0
pSV2-*neo*	0	0
pEF321-*neo*	42	8
pTK-*neo*	0	0
pSRα-*neo*	4	1

[a] 6×10^4 cells were replated in 6-cm plates and selected by 0.8 or 1.0 mg G418/ml in Dulbecco's modified Eagle's medium with 10% fetal bovine serum for 4 weeks. Colonies were counted after staining with 5% Giemsa solution (see Fig. 1).

[b] The HepG2 cells were transfected with each plasmid DNA (see Fig. 1).

plate, several hundred colonies were obtained. In fact, we could isolate many HepG2 cell clones which stably produced the hepatitis C virus (HCV) core protein using the pEF321-*neo* plasmid (data not shown). The stable expression of the HCV core pr tein was observed for at least 4 months. Although we did not examine its expression efficiency in the primary hepatocytes or the liver cells of other species, the pEF321 vector is thus useful for transient as well as stable expression of foreign genes in human liver cells.

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